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Modification of Pollutant Hydrolysis Kinetics in the Presence of Humic Substances

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JOURNAL ARTICLE

Modification of Pollutant Hydrolysis Kinetics in the Presence of Humic Substances

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■ Effects of humic substances on the kinetics of hydrolysis of the 1-octyl ester of (2,4-dichlorophenoxy)acetic acid (2,4-DOE) were investigated. In distilled water at pH 4 and above, hydrolysis is governed by second-order alkaline hydrolysis kinetics (k_2, 4.1 ± 0.9 M^{-1} s^{-1} at 24 °C). The hydrolysis kinetics are complicated by sorption of the ester to the glass container walls. System specific sorption–desorption rate constants for the ester are relatively small. Competitive equilibrium studies between glass walls, water, and "dissolved" humic substances give partition coefficients for the humic material (K_h) of 10^4–10^5. Similarly, hydrolysis studies in the presence of various humic substances give calculated partition coefficients in the range 10^4–10^5. General acid–base catalysis by dissolved humic substances, if operative at all, is masked by the sorption phenomenon. The overall effect of the humic material is an apparent decrease of the alkaline hydrolysis rate constant by a factor equal to the fraction of the ester associated with the humic substances.

Introduction

Recent reports have suggested, based on concentration–time studies, that dissolved or suspended organic materials common to natural waters can mediate the abiotic hydrolysis of organic pollutants (*1*, *2*). If these degradation processes occur at a rate competitive with other fate processes such as specific acid- or base-catalyzed hydrolysis, then it is important to include these processes in models being developed to forecast exposure concentrations of pollutants in aquatic ecosystems. Most pollutant fate models do not presently include catalytic abiotic hydrolysis processes partly because the nature, concentration, and activity of potentially important catalysts in aquatic systems have not been systematically investigated.

Reports that abiotic hydrolysis, under reaction conditions approaching those of natural waters, occurs at rates in excess of those predicted by specific acid–base-catalyzed hydrolysis (*3*) are increasingly common. These observed rate enhancements are generally based on the results of laboratory experiments using organic humic material isolated from natural waters (*1*), studies involving natural water samples brought into the laboratory (*4*), or finally, data gleaned from field observations (*5*).

Results from the first two types of experiments are, to date, the most definitive. For example, Khan (*1*) carried out experiments with the herbicide atrazine using isolated fulvic acids. Pseudo-first-order rate constants were found to increase with increasing fulvic acid concentration. Khan also observed that the catalytic rate constant at a given fulvic acid concentration was pH dependent. These results supported general acid catalysis. In a similar study, Struif and co-workers (*2*) investigated the role of fulvic acids on the degradation kinetics of a series of *n*-alkyl esters of (2,4-dichlorophenoxy)acetic acid (2,4-D). These authors concluded that adsorption to flocculated humic acids accelerated hydrolysis, based on a comparison of rate constants obtained in distilled water controls.

Results of pollutant degradation studies using natural water samples under laboratory reaction conditions also suggest that catalyzed hydrolysis might be common to natural waters. Degradation kinetic studies with the insecticide dichlorvos indicated that degradation occurred at a faster rate in natural water samples than in distilled waters (*6*). On the other hand, a detailed study of 12 pesticides in natural water samples revealed no catalytic effects when the results of heat-sterilized natural water samples were compared with distilled water samples (*4*).

Humic substances are common in natural surface waters and in interstitial waters of sediments and soils. The acidic properties of humic substances may lead to increased rates of hydrolysis through general acid–base catalysis (*7*). Conversely, their reported hydrophobic properties may actually lead to decreased hydrolysis rates through pollutant–humus association. Therefore, the *1*-octyl ester of 2,4-D, (2,4-DOE) was selected as a model compound because of its facile hydrolytic reactivity and its hydrophobicity. The experimental approach was to define the kinetics of hydrolysis of this compound in distilled water and to compare the results with similar experiments conducted in solutions of humic substances from a variety of sources.

Experimental Section

Preparation of Reagents. 2,4-DOE was prepared by reaction of the acid with 1-octanol in the presence of a catalytic amount of *p*-toluenesulfonic acid for 4 h at 80 °C. The ester was purified by fractional distillation with retention of the fraction boiling at 94–96 °C at 0.2 torr. Gas–liquid chromatographic (GLC) analysis revealed no impurities. The nuclear magnetic resonance and mass spectra were consistent with the assigned structure.

Humic substances from a variety of sources were used in this study. Many of the experiments used an extract of Aldrich humic acid, which was obtained by extracting the commercial product with aqueous NaOH at pH 8–9 for 7 days. After centrifugation at 35000*g* for 1 h, the supernatant extract was adjusted to pH 5.0 with HCl, passed through a prewashed 0.2-μm Nuclepore filter, and stored in a glass-stoppered bottle at 4 °C. The total organic carbon content of the extract was 5000 mg/L. In other experiments, humic acids and fulvic acids from Altamaha River sediments in south Georgia were used. Solutions of these substances were prepared gravimetrically, with addition of NaOH to maintain pH 8–9. After equilibration for 24 h, trace amounts of undissolved residue were removed by passing solutions through 0.2-μm Nuclepore filters. The filtered solutions were stored at 4 °C until needed. Subsequent pH adjustments were made with NaOH or HCl.

Quantitative Analysis of 2,4-DOE. Aqueous samples of 2,4-DOE solutions (3–10 mL) were extracted by addition of 1 mL of acetonitrile followed by 1 mL of isooctane, the latter of which contained 0.91 nmol of octyl 4-chlorobenzoate (PCBA-OE) as an internal standard. The samples were mixed vigorously in a vortex mixer for 60 s, with subsequent sonication for 60 min in an ultrasonic bath. This particular procedure was developed to overcome the

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very high affinity of 2,4-DOE for glass container walls. The percentage recovery of 2,4-DOE was shown to be independent of the total added 2,4-DOE over the concentration range 0.08–0.2 μM. The isooctane extract was analyzed by using a Tracor Model 222 gas chromatograph equipped with a ^{63}Ni electron capture detector. Optimum conditions were obtained on a 2.8 m × 6.4 mm glass column by using 3% SP2100 liquid phase on 80/100 Supelcoport: C_T, 215 °C; I_T, 230 °C; D_T, 250 °C. Peak:height ratios of standard solutions (2,4-DOE/PCBA-OE) were shown to be linear over the working concentration range.

Inhibition of Biodegradation of 2,4-DOE. Unless otherwise specified, all experiments were conducted under sterile conditions. All glassware, filtration equipment, prewashed membrane filters, etc., were autoclaved within 1 h of use. All solutions were filter-sterilized, by using prewashed 0.2-μm Nuclepore filters. A seres of nonsterile aqueous solutions of 2,4-DOE was treated with NaN_3 ($\sim 10^{-5}$–10^{-1} M) to determine the level of this bacterial inhibitor that maximized recovery of 2,4-DOE. On the basis of the results of these experiments, optimum recoveries were obtained at 0.0015 M NaN_3, and this concentration was used in some kinetic experiments to further inhibit any bacterial growth (*8*). Standard agar plate counting methodology was used to periodically monitor the bacterial population during each experiment (*8*). The data from experiments in which sterility was not maintained were discarded.

Water Solubility of 2,4-DOE. The water solubility of 2,4-DOE at 24 °C was determined under sterile conditions by using a specially constructed 250-mL cylindrical Pyrex vessel that was equipped with inlet and outlet stopcocks to facilitate removal of aliquots of aqueous solution without loss of sterility. An excess of 2,4-DOE (2 μmol) in 25 mL of hexane was added to the 250-mL container that was completely filled with Pyrex beads (2-mm diameter). After removal of the hexane under a stream of sterile prepurified N_2, the container was filled with 115 mL of sterile, distilled water and allowed to equilibrate. Aliquots were withdrawn periodically until an equilibrium 2,4-DOE concentration was attained in the aqueous phase. The experiment was repeated with a 5-fold increase in the amount of ester coated to the beads to ensure that the absorptive capacity of the glass had been exceeded.

Octanol–Water Partition Coefficient. In triplicate experiments, 10-mg samples of 2,4-DOE were dissolved in 2 mL of 1-octanol, equilibrated with 40 mL of water (octanol saturated), and shaken for 15 min. A 1-mL portion of the aqueous layer was extracted with 10 mL of isooctane and analyzed by GLC. A 0.5-mL sample of the octanol layer was diluted to 10 mL in hexane and analyzed by GLC. The octanol–water partition coefficient was calculated as described elsewhere (*9*).

Sediment–Water Partition Coefficient. Samples of characterized sediments (EPA 13) (OC = 3.04%) (*10*) were autoclaved and combined with filter-sterilized distilled water to prepare aqueous suspensions with sediment–water ratios (w/w) ranging from 0.001 to 0.01. The 2,4-DOE was added in acetonitrile carrier solvent (5–10 μL) to obtain 2,4-DOE concentrations ranging from 0.1 to 0.2 μM. Samples were shaken in glass-stoppered 50-mL Erlenmeyer flasks at 28 °C. At selected times, the sediment–water suspension in a single flask was centrifuged at 35000*g* for 1 h to separate the two phases. The sediment, water, and glass-container-wall samples were separately extracted and analyzed as previously described. Sediment–water partition coefficients were calculated as described elsewhere (*9*).

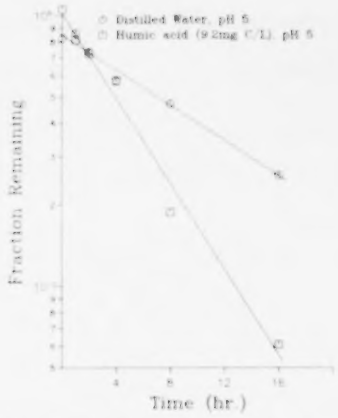

Figure 1. Effect of humic acid on rate of biodegradation of 2,4-DOE.

Table I. Water Solubility of 2,4-DOE at 24 °C

time, days	concn, μM[a]	time, days	concn, μM[a]
3	0.104 (0.108)	7	0.102 (0.083)
3	0.082 (0.133)	9	0.101
5	0.094 (0.104)	10	(0.096)

[a] The glass bead-aqueous system contained either 2 or 10 μmol of 2,4-DOE. The results from the latter system are given in parentheses.

Adsorption of 2,4-DOE to Glass Container Walls. In studies of the adsorption kinetics of 2,4-DOE to glass container walls, several replicate adsorption kinetics experiments were conducted under sterile conditions at pH 5.0 and 24 °C. In each experiment, several test tubes were filled with aqueous solutions of 2,4-DOE (0.1–0.2 μM) and analyzed individually at selected time intervals, with the aqueous phase and test-tube-wall samples being separately extracted. The quantitative effects of humic substances on the adsorption kinetics were examined under identical experimental conditions, with the aqueous phase being one of the humic acid or fulvic acid solutions that were previously described.

Hydrolysis Kinetics of 2,4-DOE. Hydrolysis kinetic experiments were conducted in the test tubes that were used in the adsorption experiments described above. The 2,4-DOE was added in tetrahydrofuran (5–10 μL) carrier solvent to either distilled water or selected humic acid solutions (0.1–0.2 μM) with pH values ranging from 8 to 11. At selected intervals throughout an experiment, a test tube and its contents were extracted either together or separately as previously described. Accordingly, in some experiments only the change in "total" 2,4-DOE concentration was observed, whereas in other experiments the rates of change of both "dissolved" and "adsorbed" 2,4-DOE concentrations were determined.

Results

Biodegradation of 2,4-DOE. The effect of Aldrich humic acid extract (9.2 mg C/L) on the rate of biodegradation of 2,4-DOE is exemplified in Figure 1, in which the observed rate constant for disappearance of 2,4-DOE from nonsterile distilled water is significantly increased upon addition of humic acid. This apparent rate enhancement by humic acid (cell count of 4.4×10^9 cells/L) is *not* observed in sterile solutions (see subsequent discussion).

Physical Constants. The results of the water solubility experiments are summarized in Table I. Because the

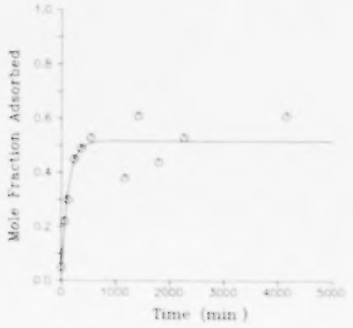

Figure 2. Kinetics of adsorption of 2,4-DOE to glass container walls.

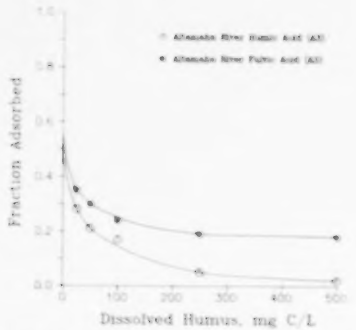

Figure 3. Effect of dissolved humus on adsorption of 2,4-DOE to glass container walls.

Table II. Adsorption–Desorption Rate Constants for the 2,4-DOE to Glass Container Walls

pH	n[a]	$10^3 k_f$, min^{-1}	$10^3 k_r$, min^{-1}	ESS[b]
5.0[c]	10	2.8	1.5	0.0836
5.2[d]	11	2.6	2.7	0.0994
5.3[c]	11	4.2	3.9	0.0448

[a] n = number of data points. [b] ESS = $\Sigma(Y_{calcd} - Y_{expt})^2$. [c] Initial concentration was 0.2 μM. [d] Initial concentration was 0.1 μM.

Table III. Partition Coefficients of 2,4-DOE to Humic Substances: Competitive Equilibrium Results

humic substance	log K_h
Aldrich humic acid extract	4.4 (±0.1)
sedimentary humic acid (A3)	5.1 (±0.1)
sedimentary fulvic acid (A3)	3.8 (±0.1)
sedimentary humic acid (A6)	4.2 (±0.1)
sedimentary fulvic acid (A6)	3.8 (±0.1)

results were independent of total added 2,4-DOE, the aqueous phase was assumed to be saturated. The overall average of all the measurements yields a water solubility of 0.101 ± 0.014 μM.

For the octanol–water partition coefficient, the average value of nine measurements is $3.7 \times 10^5 \pm 54\%$ (log K_{ow} of 5.6). This value is in agreement with the value of 5.7 predicted from the water solubility and the correlation between the octanol–water partition coefficient and water solubility as considered by Banerjee et al. (*11*).

This average value of the sediment–water partition coefficient (normalized to organic carbon) (K_{oc}) for the 2,4-DOE in the processed sediment (EPA 13) is $2.0 \times 10^5 \pm 58\%$ (log K_{oc} of 5.3). This value agrees with the calculated value of log K_{oc} of 5.4 obtained from the experimental K_{ow} and the correlation of K_{oc} with K_{ow} (*12*).

Adsorption of 2,4-DOE to Glass Container Walls. Typical results from the quantitative adsorption experiments are given in Figure 2. If a low extent of surface coverage is assumed, the equilibrium distribution of 2,4-DOE between aqueous solution and glass container walls depends on the surface–volume ratio of the test tubes (ρ_g) and the partition coefficient (K_g):

$$X_g = \rho_g K_g / (1 + \rho_g K_g) \quad (1)$$

where X_g is the fraction of 2,4-DOE adsorbed on glass container walls at equilibrium. The rate of approach to equilibrium can be described in terms of the adsorption and desorption rate constants:

$$\text{2,4-DOE(aq)} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} \text{2,4-DOE(ads)} \quad (2)$$

and

$$\rho_g K_g = k_f / k_r \quad (3)$$

With data from three experiments at approximately pH 5 (see Table II), the following values can be calculated by simultaneous solution of the rate equations: $k_f = 3.17 \times 10^{-3}$ min^{-1} (±27%), $k_r = 2.67 \times 10^{-3}$ min^{-1} (±45%), and $\rho_g K_g \approx 1.2$. Values for k_f and k_r were obtained from experiments using initial concentrations of 0.2 μM (pH 5.0 and 5.3, Table II) and 0.1 μM (pH 5.2, Table II) and are in good agreement. Thus, due to the sorptive capacity of the glass, it was possible to work at 0.2 μM and not supersaturate the aqueous phase but, at the same time, enhance analysis by working at the higher concentrations.

In the presence of "dissolved" humic substances (25–500 mg of C/L), the extent of adsorption of 2,4-DOE to glass container walls is significantly diminished (see Figure 3). This phenomenon is consistent with the association of 2,4-DOE with "dissolved" humic substances (passed through a 0.2-μm Nuclepore filter) to yield a moiety that does not detectably adsorb to glass. In a simple conceptual model of this interaction, "dissolved" humic substances are treated as a separate phase into which 2,4-DOE is partitioned. The equilibrium distribution of 2,4-DOE between water and humic substances can be described then as a function of the concentration of humic substances (ρ_h, (g of C/g of H_2O)) and the appropriate partition coefficient (K_h). The equilibrium mole fraction of 2,4-DOE adsorbed on glass container walls is thus given by

$$X_g = \rho_g K_g / (1 + \rho_g K_g + \rho_h K_h) \quad (4)$$

The best estimate of K_h for each humic substance used in these experiments was obtained (rearranging eq 4 to eq 5) by a least-squares fit of the data to eq 5 with the es-

$$\rho_g K_g (1 - X_g)/X_g - 1 = K_h \rho_h \quad (5)$$

timated value of 1.2 used for $\rho_g K_g$. The results are given in Table III. It should be noted that, based on this simple model, a plot of $1/x_g$ vs mg of C/L should be linear. However, if the data in Figure 3 are plotted accordingly, there is some deviation from linearity. Although the reason for this is not understood, this simple model is useful in describing the effect of sorption.

Hydrolysis Kinetics of 2,4-DOE. The base-catalyzed hydrolysis of 2,4-DOE was studied in the pH range 8–11. Typical plots of log ($[2,4\text{-DOE}]_t/[2,4\text{-DOE}]_0$) vs. time are given in Figure 4.

The linear relationship expected from pseudo-first-order kinetics is clearly not observed at the higher pH. Previous discussion has indicated that, at equilibrium, approximately 55 mol % of 2,4-DOE should be adsorbed on con-

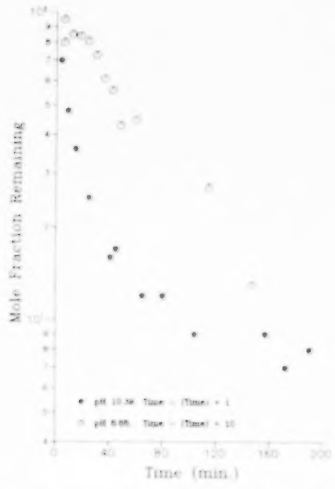

Figure 4. Alkaline hydrolysis of 2,4-DOE in distilled water.

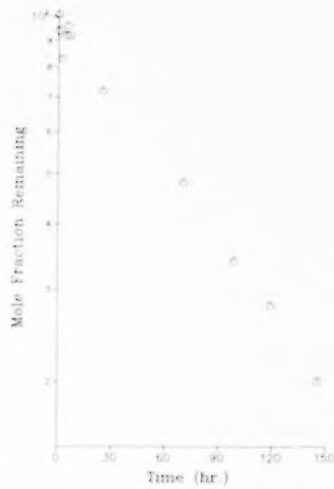

Figure 5. Effect of humic acid on alkaline hydrolysis of 2,4-DOE.

Table IV. Second-Order Alkaline Hydrolysis Rate Constant for 2,4-DOE in Water at 24 °C

pH	n^a	k_2, s^{-1} ($L\ mol^{-1}$)	ESS[b]
8.88	12	4.8	0.075
10.04	15	3.1	0.344
10.39	13	4.4	0.044

[a] n = number of data points. [b] $ESS = \Sigma(Y_{calcd} - Y_{expd})^2$.

Table V. Partition Coefficients of 2,4-DOE to Humic Acid: Kinetic Transformation Results

pH	n^a	log K_h	ESS[b]
8.42	16	3.9	0.077
9.44	13	4.8	0.071
9.47	11	4.9	0.027

[a] n = number of data points. [b] $ESS = \Sigma(Y_{calcd} - Y_{expd})^2$.

tainer walls, thus reducing the observed rate constant proportionately. A correction of the pseudo-first-order equation for equilibrium adsorption, however, would still yield a linear relationship between ln [2,4-DOE] time. The simplest model that adequately explains both the linear and nonlinear curves in Figure 4 simply treats the adsorption–desorption processes as relatively slow, potentially rate-limiting reactions. This treatment is consistent with the magnitudes of the previously discussed rate constants for adsorption and desorption (3.17×10^{-3} and 2.67×10^{-3} min^{-1}, respectively). The appropriate equations are

$$\text{2,4-DOE(aq)} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} \text{2,4-DOE(ads)} \qquad (6)$$

$$\text{2,4-DOE(aq)} + OH^- \xrightarrow{k_2} \text{products} \qquad (7)$$

the products of reaction being 2,4-D and octanol.

With the previously determined values for k_f and k_r, k_2 values were determined by using a curve-fitting routine that minimized the residual sum of squares to obtain the optimum fit for each data set. The results for three experiments are tabulated in Table IV. The average value for k_2 is 4.1 $M^{-1}\ s^{-1}$ (±22%). This constant is in good agreement with the value of 3.7 $M^{-1}\ s^{-1}$ calculated with data for the methyl ester of 2,4-D and the structure–activity relation for acetic acid esters (*13*). The validity of this treatment is clearly linked to the validity of our assumption that k_f and k_r are pH independent. Insufficient data were available to warrant the use of a more complex model.

Rates of alkaline hydrolysis were dramatically decreased in the presence of Aldrich humic acid extract. Since the earlier discussion indicates that 2,4-DOE partitions into "dissolved"humic substances, the decrease in the alkaline hydrolysis rate may be attributable to the same phenomenon, assuming that humic-bound 2,4-DOE is unreactive. The kinetic model given in eq 6 and 7 is expanded to include a rapid, reversible partitioning of 2,4-DOE between water and humic carbon:

$$\text{2,4-DOE(aq)} \underset{}{\overset{K_h}{\rightleftharpoons}} \text{2,4-DOE(humus)} \qquad (8)$$

With the previously determined values for k_f, k_r, and k_2, K_h values were estimated from kinetic data obtained in Aldrich humic acid extract solutions (500 mg of C/L), by using the aforementioned curve-fitting routine. A typical pseudo-first-order plot is given in Figure 5, and calculated K_h values are given in Table V. Although the value at pH 8.42 is somewhat low, it should be noted that only about 15% reaction occurred during the experiment, making it difficult to determine K_h. At this time, insufficient data are available to determine whether the apparent increase in K_h with increasing pH is real or merely an artifact. At the present time, it is assumed that K_h is pH independent and that log $K_h \approx 4.8$. This value is in agreement with the value of log $K_h = 4.4$ derived from the adsorption experiments (see earlier discussion).

In the aquatic environment, even at relatively low dissolved organic carbon (DOC) concentrations, a significant fraction of 2,4-DOE is expected to be associated with humic substances. For example, at a DOC of 10 mg/L, with $4.4 \leq \log K_h \leq 4.8$, approximately 20–40% of 2,4-DOE is humic associated.

The log K_h value for association of 2,4-DOE with "dissolved" humic acid (log $K_h = 4.8$) is close to the log K_{oc} for adsorption of 2,4-DOE onto sedimentary organic carbon (log $K_{oc} = 5.3$). This agreement supports the adsorption model used in this study, in which "dissolved" humus is treated as a nonaqueous phase. Additional support is obtained by comparison of log K_h with the octanol–water partition coefficient (log $K_{ow} = 5.6 \pm 0.3$) of 2,4-DOE. Recent evidence for association of *p,p'*-DDT with aquatic humus (*14*) also indicates that log K_h values are comparable to log K_{oc} values and are somewhat smaller than log K_{ow} values (4.6, 5.4, and 6.2, respectively) (*15*, *16*).

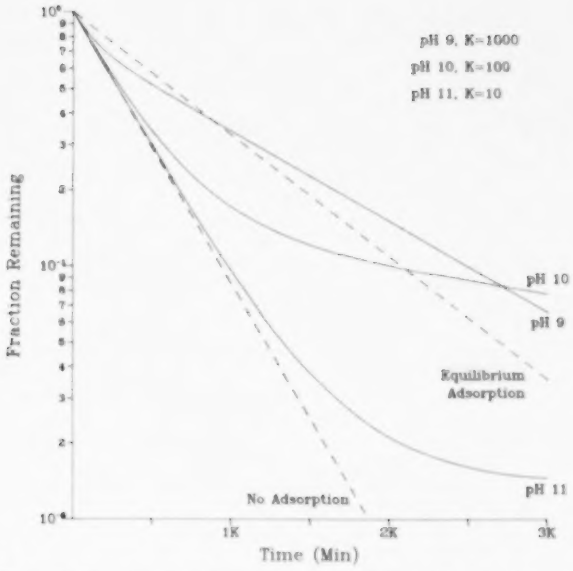

Figure 6. Non-steady-state model for overall rate of hydrolysis.

The observation that humic substances decrease the rate of alkaline hydrolysis of 2,4-DOE contradicts the results of Struif et al. (2), who reported that humic acid suspensions catalyzed the rates of hydrolysis of several esters of 2,4-D, a phenomenon that they attributed to general acid–base catalysis by humic acid. In the present study, acceleration of disappearance rates by humic acid was observed only in *nonsterile* solutions (see previous discussion). Because Struif et al. (2) did not explicitly or implicitly state that sterile solutions were used, it seems likely that the rate enhancement they reported was, in fact, biodegradation rather than general acid–base catalyzed hydrolysis.

The non-steady-state equations used to analyze the kinetic data in this study can be used to illustrate the range of possible "pseudo-first-order" kinetic plots that can be obtained whenever adsorptive and desorptive processes are competitive with hydrolysis reactions. With the "best" estimates of $k_f = 3.17 \times 10^{-3}$ min^{-1}, $k_r = 2.67 \times 10^{-3}$ min^{-1}, and $k_2 = 246$ M^{-1} min^{-1}, the plots of Figure 6 were calculated in the pH range 9–11 with the abcissa (time) scale being decreased by a factor of 10 for each increase of 1 pH unit.

In the absence of adsorptive and desorptive processes, the lower dashed line would be observed at all pH values. Likewise, if adsorption and desorption were kinetically fast (relative to alkaline hydrolysis), the upper dashed line would be observed at all pH values. The actual predictions from the observed rate constants are clearly quite sensitive to pH as shown by the solid lines. As pH is gradually increased, the system slowly changes from one where alkaline hydrolysis is rate limiting and the adsorption–desorption process is at equilibrium to a system where alkaline hydrolysis, adsorption, and desorption are roughly comparable. Under such conditions, the reaction rate sooner or later becomes rate limited by the desorption reaction. This situation is exemplified at pH 10 (see Figure 7). At very high pH values, the hydrolysis reaction is so fast that essentially no adsorption (and, hence, no desorption) occurs. The rate-limiting step is once against the hydrolysis reaction.

The effect of partitioning of 2,4-DOE into "dissolved" humic substances on the rate of 2,4-DOE hydrolysis is illustrated in Figure 8 at pH 10 for a range of concentra-

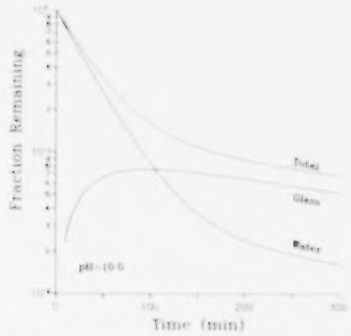

Figure 7. Non-steady-state model of adsorption, desorption, and hydrolysis.

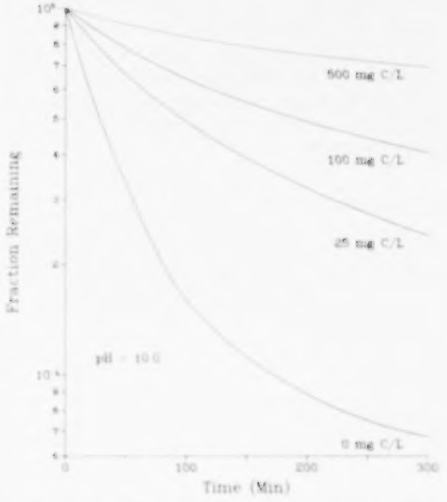

Figure 8. Effect of partitioning on the hydrolysis of 2,4-DOE at pH 10 with varying amounts of "dissolved humic substances".

tions of humic substances. Even at environmentally realistic concentrations, humic substances should significantly modify the kinetics of alkaline hydrolysis of 2,4-DOE.

Although the general importance of adsorption and desorption of sclutes to and from container walls is not known, it is apparent that those processes, whenever they are significant, greatly complicate the acquisition and analysis of kinetic data. Preliminary studies of container effects on reaction kinetics are certainly recommended to avoid misinterpretation of kinetic data.

Summary and Conclusion

The second-order alkaline hydrolysis rate constant of 2,4-DOE was determined in aqueous solution at 24 °C ($k_2 = 4.1$ M^{-1} s^{-1}). Analysis of the kinetic data was greatly complicated by the relatively slow, reversible adsorption of 2,4-DOE onto glass test tube walls, which resulted in marked deviations from the pseudo-first-order reaction rate that had been anticipated.

On the basis of these observations, partitioning of hydrophobic compounds between aqueous solutions and glass container walls cannot generally be assumed to be fast. Rapid, efficient extraction of the glass-adsorbed fraction of such compounds requires quite vigorous mixing of the aqueous sample and the extracting solvent.

In the presence of "dissolved" humic substances, significant decreases in both the equilibrium mole fraction of 2,4-DOE adsorbed to glass and the apparent alkaline

hydrolysis rate constant of 2,4-DOE were observed. These observations are mutually consistent with a rapid, reversible partitioning of 2,4-DOE between water and "dissolved" humic substances. The humic-bound 2,4-DOE is not adsorbed to glass and is protected from alkaline hydrolysis. On the basis of this model, with aquatic humus concentrations expressed as g of C/g of H_2O, the distribution coefficient of 2,4-DOE between water and aquatic humus (K_h) was calculated from both the glass adsorption data and the alkaline hydrolysis data, yielding log K_h values of 4.4 and 4.8, respectively.

From the magnitude of the distribution coefficient of 2,4-DOE between water and "dissolved" humic substances, a significant interaction is generally expected for hydrophobic compounds in natural waters. Rates of alkaline hydrolysis, volatilization, etc., would be expected to be reduced in proportion to the fraction of a hydrophobic solute that is associated with humic substances.

If any general acid–base catalysis of the hydrolysis reaction is attributable to humic substances, that contribution is completely masked by the partitioning phenomenon, which strongly decreases the rate of alkaline hydrolysis of 2,4-DOE.

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